

Telomere loss limits the rate of human epithelial tumor formation.

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Most human carcinomas exhibit telomere abnormalities early in the carcinogenesis process [1–3] suggesting that crisis caused by telomere shortening may be a necessary event leading to human carcinomas [4]. Epidemiological records of the age at which each patient in a population develops carcinoma are known as age-incidence data; these provide a quantitative measure of human tumor initiation and dynamics [5]. If crisis brought on by telomere shortening is necessary for most human carcinomas, it may also be the rate limiting step. To test this, we compared a mathematical model in which telomere loss is the rate limiting step during carcinogenesis with age-incidence data compiled by the Surveillance, Epidemiology and End Results (SEER) program [6]. We found that this model adequately explains the age-incidence data. The model also implies that two distinct paths exist for carcinoma to develop in prostate, breast, and ovary tissues. We conclude that a single step, crisis brought on by telomere shortening, limits the rate of formation of human carcinomas.

Introduction

Crisis brought on by shortening telomeres, or senescence, may be a necessary step in epithelial carcinogenesis. A model of cellular senescence suggests that cells reach their replicative limit, the Hayflick limit [7], as the length of their telomeres shorten [8,9]. Upon reaching this limit, cells face a crisis. A few cells, probably those that have acquired certain mutations, are able to overcome this crisis and continue replicating [10], while most others cannot survive and perish. The surviving cells, replicating without functional telomeres, exhibit massive genomic instability. Genomic instability is also characteristic of epithelial cancers [4]. The epithelial carcinogenesis process exhibits telomere abnormalities both early and often [1–3]. Hence, crisis caused by telomere shortening may be a necessary event in epithelial carcinogenesis.

If crisis is necessary for epithelial carcinogenesis, it may also be the rate limiting step. Identifying the rate-limiting step is key to delaying the onset of carcinogenesis. If one reduces factors that are not rate-limiting, no significant change in onset will result.

Surveillance networks record the age at which carcinoma is diagnosed in patients. These cover just over 25% of the United States population. With census data, this can be converted to a rate. We use this data to test the hypothesis that crisis brought upon by reaching cellular senescence is the rate limiting step in carcinoma formation.

Long ago [11,12], it was pointed out that if r mutations are required before carcinoma develops, if each mutation occurs at a constant rate, and if cells with less than r mutations have no growth advantage, then the age-specific incidence of carcinoma should be $I(t) = kt^{r-1}$ [5]. Although, this model has evolved [13,14] to include effects such as an intermediate growth advantage, its basic conclusion remains. When this model is applied to colon carcinoma age-incidence data collected by cancer registries, it leads to the conclusion that four to six mutations are required to transform a normal cell into a malignant one. This conclusion is now widely accepted [15].

However, this model fails to explain several anomalies with the age-incidence data. Breast carcinoma incidence data does not follow this model. (This has been explained by a model suggesting that breast tissue ages at a different rate than calendar time [16]). Prostate carcinoma incidence increase much more rapidly with age than most others, implying that 20 to 30 mutations are required for transformation. Also, the rate of increase in the incidence for several carcinomas begins to slow or even drop at advanced ages [17]. None of these phenomena can be explained with the current model.

Widespread biochemical and medical imaging screening programs implemented in the United States during the past two decades have led to earlier and more complete diagnosis of carcinoma. This, in turn, has led to dramatic differences in the age-incidence data collected by cancer registries. This data presents an increasingly more accurate picture of when carcinoma actually develops, as opposed to when it becomes fatal.

Methods

Mathematical Model We constructed a mathematical model to describe the expected age-incidence of carcinoma. The mathematical model is based upon the biological model that carcinoma only develops after the onset of crisis. The assumption is that epithelial carcinogenesis has a single rate limiting step and that step occurs when telomere shortening has reached its limit. The simplest model would be that carcinoma develops in every

member of the population at exactly the same age. However, due to the heterogeneous population living in a heterogeneous environment, we would expect that this statement generalizes to the mathematical statement that the age-incidence follows a normal distribution.

Although it is not possible to rigorously justify its use here, the normal distribution is ubiquitous in nature [18]. Its finite value at negative ages may present theoretical objections to its application in this instance, but as long as the mean is large enough and standard deviation small enough (which is true in all cases here), these theoretical objections have no practical grounding.

The age-incidence, $I(t)$, of carcinoma for the model is given by

$$I(t) = \alpha \left(\frac{N(t, \tau, \sigma)}{1 - \int_0^t N(s, \tau, \sigma) ds} \right), \quad (1)$$

where α represents the number of people susceptible to the cancer. $N(t, \tau, \sigma)$ is the well-known normal distribution, also called the Gaussian distribution, or bell-shaped curve. It is given by

$$N(t, \tau, \sigma) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\left(\frac{(t-\tau)^2}{2\sigma^2}\right)}. \quad (2)$$

The factor in the denominator of Equation 1, $1 - \int_0^t N(s, \tau, \sigma) ds$, adjusts for the number of people who already have acquired carcinoma. For most carcinomas, it is very close to one. It ranges from 1.0 to 0.94 for colon carcinoma.

If carcinoma can develop by two distinct paths, the observed age-incidence will be a linear combination of two independent functions,

$$I(t) = \alpha_1 \left(\frac{N(t, \tau_1, \sigma_1)}{1 - \int_0^t N(s, \tau, \sigma) ds} \right) + \alpha_2 \left(\frac{N(t, \tau_2, \sigma_2)}{1 - \int_0^t N(s, \tau, \sigma) ds} \right). \quad (3)$$

SEER Data We tested the models, Equation 1 and Equation 3, by fitting them to age-incidence data from different carcinomas. The age-incidence data were recorded by the SEER 12 registries [6] and the rates were age-adjusted to the 2000 US population as measured by the census. The SEER registries have compiled cancer incidence information on a large representative sub-population of US residents since 1975. From this database, we selected patients diagnosed in 2000 with carcinoma in the indicated tissue. This excludes the small number with other types of cancers, sarcomas for instance, which probably arise through a different process. The calculation of confidence intervals are based upon the method of Fay and Feuer [19].

We chose to analyze data for patients who were diagnosed in the same year (2000), rather than those born in the same year (a birth-cohort). Different factors could distort either data set. Birth-cohort analysis is significantly distorted by changes in medical practice and diagnostic technology. On the other hand, changes in environmental carcinogens may distort the data presented here. Detection technology for the carcinomas presented here have dramatically improved over the past 50 years. Hence, we focus on patients diagnosed in a single year, while recognizing that their environmental exposure may be different.

Model Fitting The model was fit to the data by minimizing chi-squared, χ^2 [20],

$$\chi^2 = \sum_{k=1}^{17} \frac{(O_k - E_k)^2}{E_k}, \quad (4)$$

where O_k is the observed number of cases of cancer and E_k is the expected number of cases of cancer in each of the 17 age ranges. The 18th age group includes all those diagnosed with cancer at or above 85 years of age. This age group was not included in calculating the minimization, since it is unbounded. The expected number of cases, E_k , was obtained by multiplying the age-corrected incidence function $I(t)$, evaluated at the mid-point of the age group range, by the population under surveillance in that age range. The parameters α , τ , and σ were varied to minimize χ^2 . This optimization used the Generalized Reduced Gradient algorithm [21] to perform the minimization. Estimates on the errors of the parameters were obtained by fitting five independent data sets (data from five different years, 1996 – 2000) and reporting the mean and standard error of these measurements.

Results

Age-incidence data from carcinomas originating in the colon, stomach, pancreas, and esophagus are consistent with the single path model, Equation 1. In each case, we found an excellent fit between the model and the actual age-incidence data, see Figure 1 for the fit to colon carcinoma data, and the Supplemental Figures for the others.

Breast, ovarian, and prostate carcinoma are consistent with the existence of two paths in the model, Equation 3. In ovarian carcinoma, see the Supplemental Figures, these two paths have dramatically different mean times, 22 years and 78 years, and the early version is over a hundred times rarer. While in prostate carcinoma, see the Supplemental Figures, the two mean times are relatively close, 68 and 80 years. In breast carcinoma, see Figure 2, the two versions of the disease occur far enough apart, 52 and 76 years and the population-at-risk is similar enough, 5% and 19%, so that the two populations can be discerned. We also examined other non-epithelial cancers. These generally are not consistent with the presented model. In Table 1, we present the fitted parameters for each carcinoma along with error estimates.

Discussion

The results presented here indicate that the age-incidence data is consistent with a model in which cellular senescence is the rate-limiting step in the development of carcinoma.

The mathematical model has three parameters, and each has a well defined meaning. The first parameter, the mean time, τ , indicates the average time, measured from birth, for the formation and detection of the cancer. One would expect that environmental influences can affect this parameter, and that may be one explanation for the variation of times observed in the population. The second parameter, the standard deviation of the time, σ , quantifies this variation in the population. This variation can be attributed to either intrinsically random processes, genetic variation, or environmental differences within the population. The final parameter, the susceptible population, α , describes the fraction of the total population susceptible to this carcinoma. This parameter is always greater than

Table 1: Three parameters describe the age-incidence curve for each carcinoma. The first parameter, α , represents the fraction of the population susceptible to the disease. In the case of ovarian and breast cancer it is the fraction of women; while for prostate carcinoma it represents the fraction of men. The second parameter, τ , is the mean time (in years) to develop the disease. The final parameter, σ , is the standard deviation of the time (in years).

Tissue		Susceptible population (α)	Mean time (τ) (years)	S.D. time (σ) (years)
Colon		20.0(± 1.3)%	90.8(± 1.0)	18.8(± 0.3)
Stomach		5.2(± 0.7)%	101.0(± 2.6)	22.2(± 0.8)
Pancreas		3.2(± 0.2)%	87(± 1.6)	17.1(± 0.7)
Esophagus		1.0(± 0.1)%	80(± 2.8)	15.3(± 1.1)
Breast	Early	5.1(± 0.7)%	52.0(± 0.5)	9.4(± 0.1)
	Late	17.2(± 1.4)%	75.3(± 0.6)	13.4(± 0.9)
Prostate	Early	12(± 1)%	67(± 1)	8.4(± 0.2)
	Late	16(± 1)%	79(± 1)	9.4(± 1.3)
Ovary	Early	0.02(± 0.004)%	23(± 1.3)	5.4(± 0.7)
	Late	3.1(± 0.3)%	78(± 2.4)	21.1(± 1.1)

the actual fraction who develop the disease, since some may die before developing the disease.

Two surprising conclusions arise from this analysis. First, two distinct paths exist for the development of breast, ovarian, and prostate carcinomas. Second, substantially less than 100% of the population is susceptible to developing any specific carcinoma.

Other studies also suggest that two distinct breast carcinomas exist. Early-onset breast carcinoma is already a recognized subclass of the disease, typically being described as occurring in women before the age of 35 [22]. Our results indicate significant overlap between the early and late onset forms, and age itself is insufficient to determine whether a woman has one form of the disease or another. However, most cases (about 80%) diagnosed before the age of 35 will be early-onset. Published work concludes that early-onset disease is biologically distinct [23, 24] from the late onset version and also a more potent form of the disease [25]. Hence, our conclusion is consistent with the modern view of breast cancer.

An alternative explanation for the atypical age-incidence curve of breast carcinoma has been proposed [16]. This explanation suggests that breast tissue does not age linearly, but rather as a function of hormonal levels. If this “breast age” is properly considered, the age-incidence of breast cancer more closely follows that typically seen in other cancers. This is the currently accepted view [26]. In contrast, our model explains the breast cancer age-incidence based on two widely accepted concepts: genomic instability, and the presence of two distinct forms of the disease.

The age-incidence data, viewed in the context of this mathematical model, implies that only a subset of the population is susceptible to developing the disease. Similar conclusions have been drawn by others. Peto and Mack [27] based their conclusion on

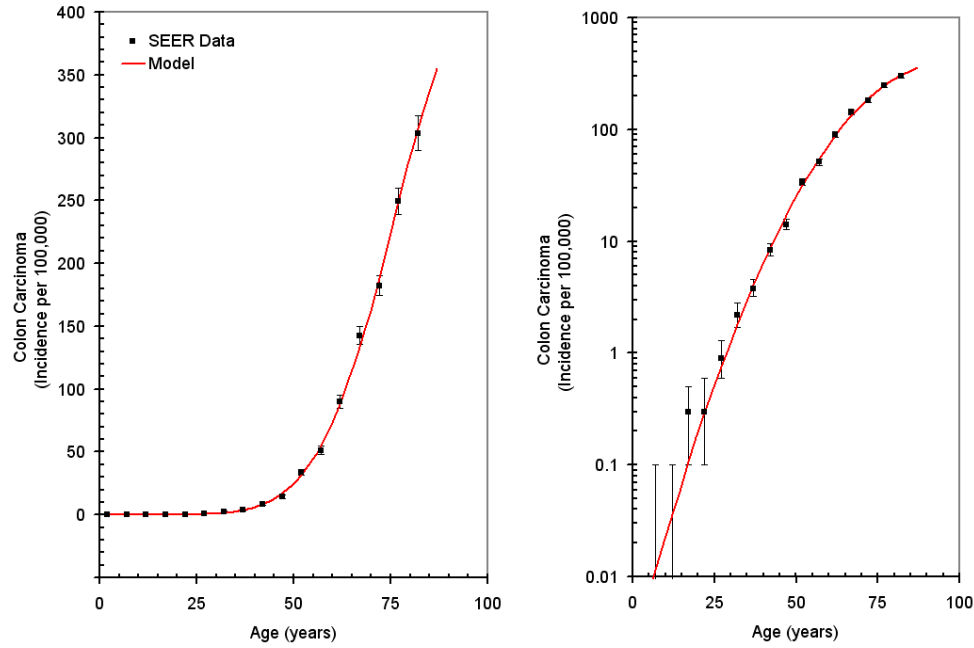


Figure 1: The observed age-incidence of colon carcinoma (A) and breast carcinoma (B) compared to the model. The left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. The logarithmic scale better represents lower incidence levels, while the linear scale better represents higher incidence levels. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.

a study of the incidence of breast cancer in monozygotic twins. A similar study [28] performed on a more extensive dataset suggests that this result is an artifact. However, the conclusion is supported by a segregation analysis [29] and by this work. Thilly's group [30, 31] has extended the Nordling, Armitage-Doll model to include the processes of mutation, cell growth and turnover while also accounting for heterogeneity of both genetic factors and environmental exposure. They concluded that the sub-population at risk for colon carcinoma was about 42% and has been invariant for over a century.

Different mechanisms could explain the existence of a minority that is susceptible to carcinoma. For instance, members of this minority may have inherited susceptibility. Although we can rule this out for high-penetrance alleles, since by definition sporadic carcinoma does not show familial aggregation, it is thought that low-penetrance alleles [32] may be responsible for this. An alternative is that membership in this minority may indicate the acquisition of a somatic mutation early in life as proposed by Frank and Nowak [33]. They reason that most carcinomas could result from somatic mutations occurring within stem cells during development. Finally, it may be an indication of This mechanism is consistent with our findings.

Although some forms of carcinoma are clearly associated with environmental exposure, the mechanism causing this has not been clearly determined. The most widely held hypothesis, that environmental carcinogens induce point mutations thus causing cancer, is not supported by observation [34]. The analysis presented here suggests an alternative.

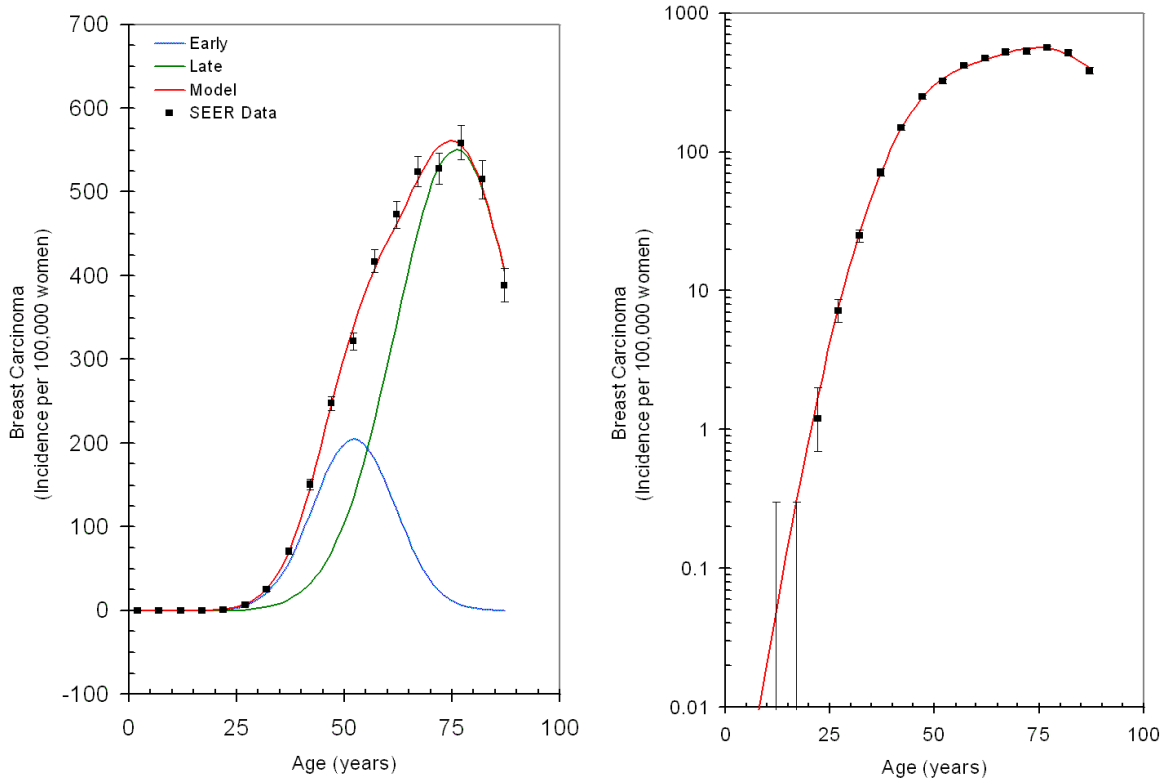


Figure 2: The age-incidence of breast carcinoma. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model. The age-incidence data can only be explained if two distinct tumorigenesis paths exist. One, the early-onset path, is represented by the blue line, and the other, the later-onset path, by the green line. In this case, the predicted incidence is the sum of the two, represented by the red line.

If environmental carcinogens cause more rapid cellular proliferation, the Hayflick limit would be reached at a younger age, and carcinoma could develop earlier. Thus environmental carcinogens would lead to increased rates of carcinoma.

The conclusions here are based on the validity of the SEER data. This is the most comprehensive dataset available. Audits are regularly conducted to ensure the completeness and accuracy of the dataset. However, others [35] have pointed out that the SEER population numbers do not exclude those patients who cannot contract the disease. For instance, women who have had hysterectomies cannot contract ovarian cancer. Approximately 0.5% of women have hysterectomies annually [36]. Thus, the ovarian carcinoma incidence-rate is likely to be systematically underestimated in the SEER data, particularly in the elderly. We estimate this systematic error to be as large as 20-30%. Although this would affect the numbers reported in Table 1, it is unlikely to affect the overall conclusions. Hysterectomies are the most common surgical procedure, hence we expect systematic errors due to this effect to be much less in other carcinomas.

In conclusion, we found that the formation of human carcinomas is limited by a single step, consistent with a model in which crisis brought on by telomere shortening is the key

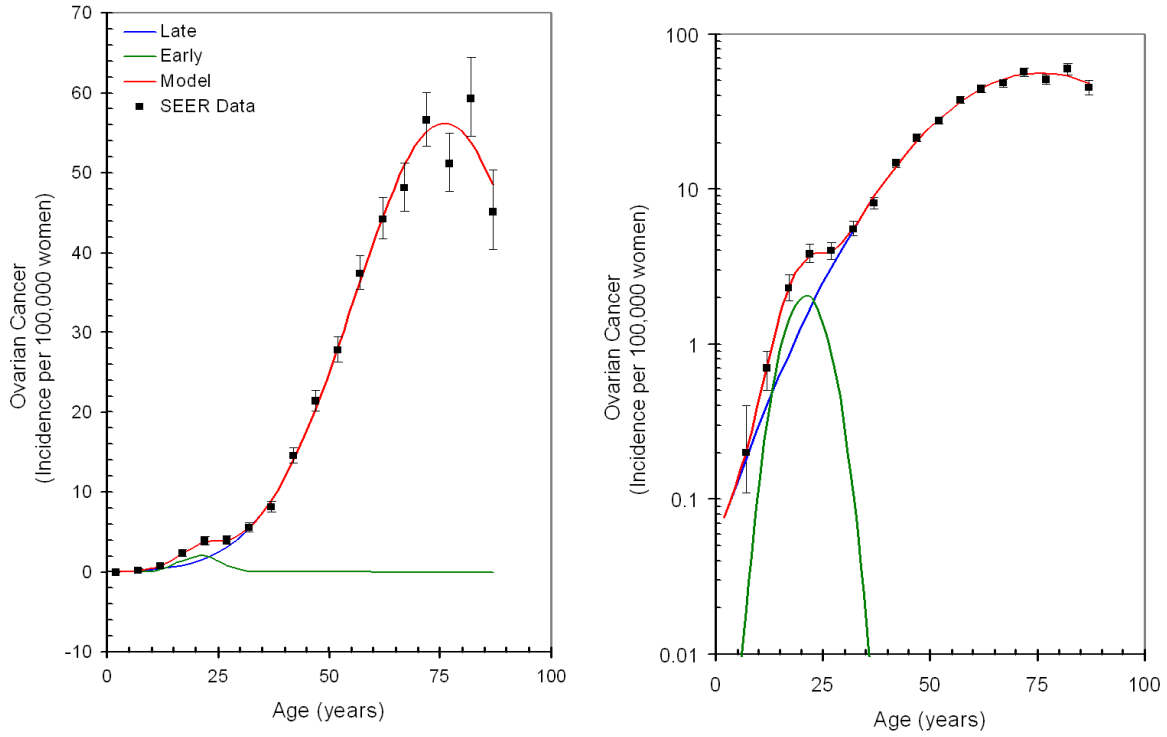


Figure 3: The age-incidence of ovarian cancer. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.

step. Furthermore, if this is true, then carcinomas predominantly develop in a susceptible minority of the population and carcinoma of the breast, ovary, and prostate can occur through two distinct paths.

Acknowledgments

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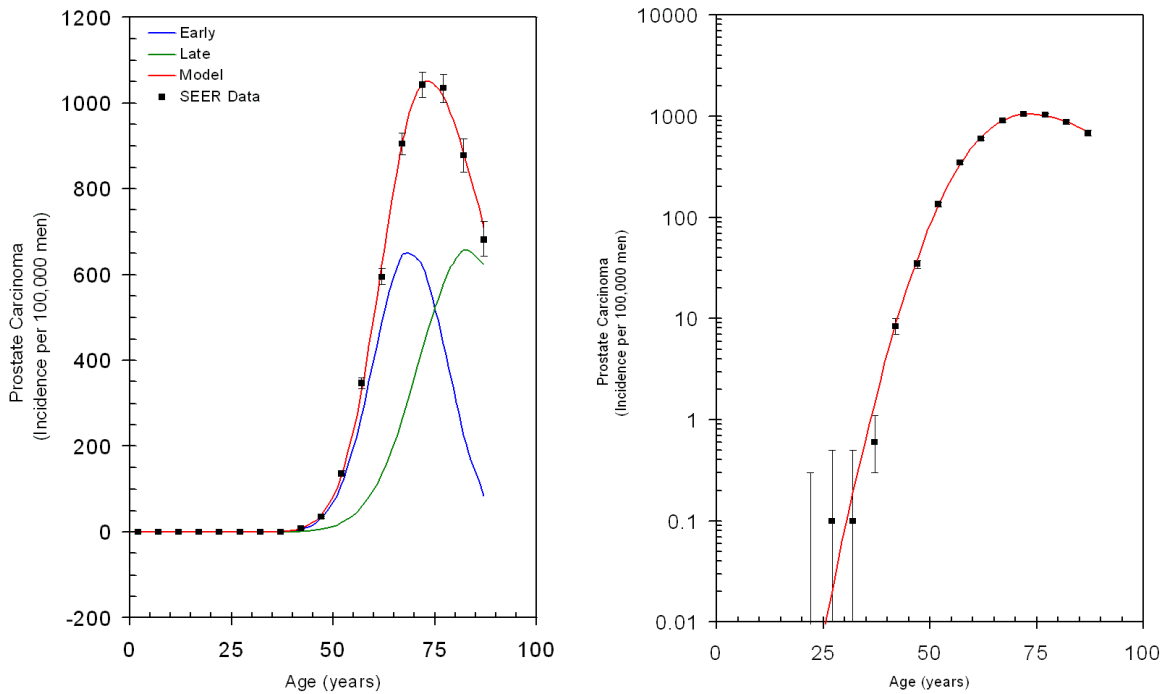


Figure 4: Supplemental Figure. The age-incidence of prostate carcinoma. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.

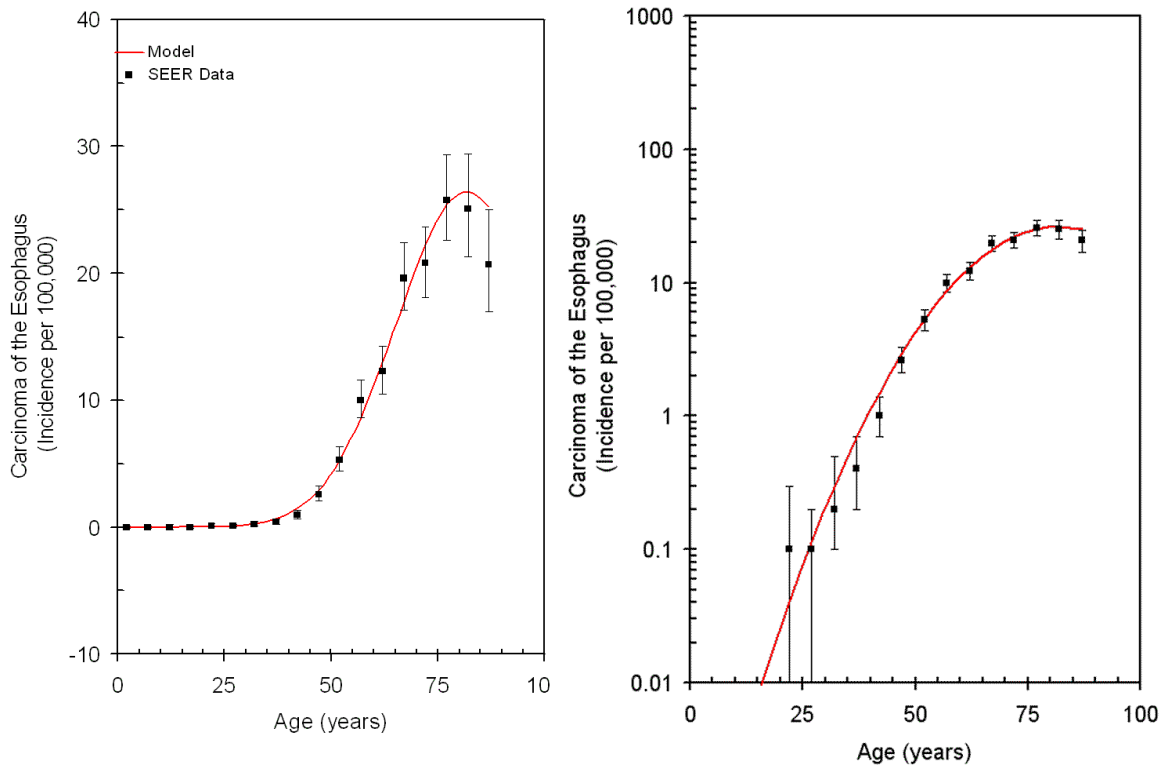


Figure 5: Supplemental Figure. The age-incidence of carcinoma of the esophagus. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.

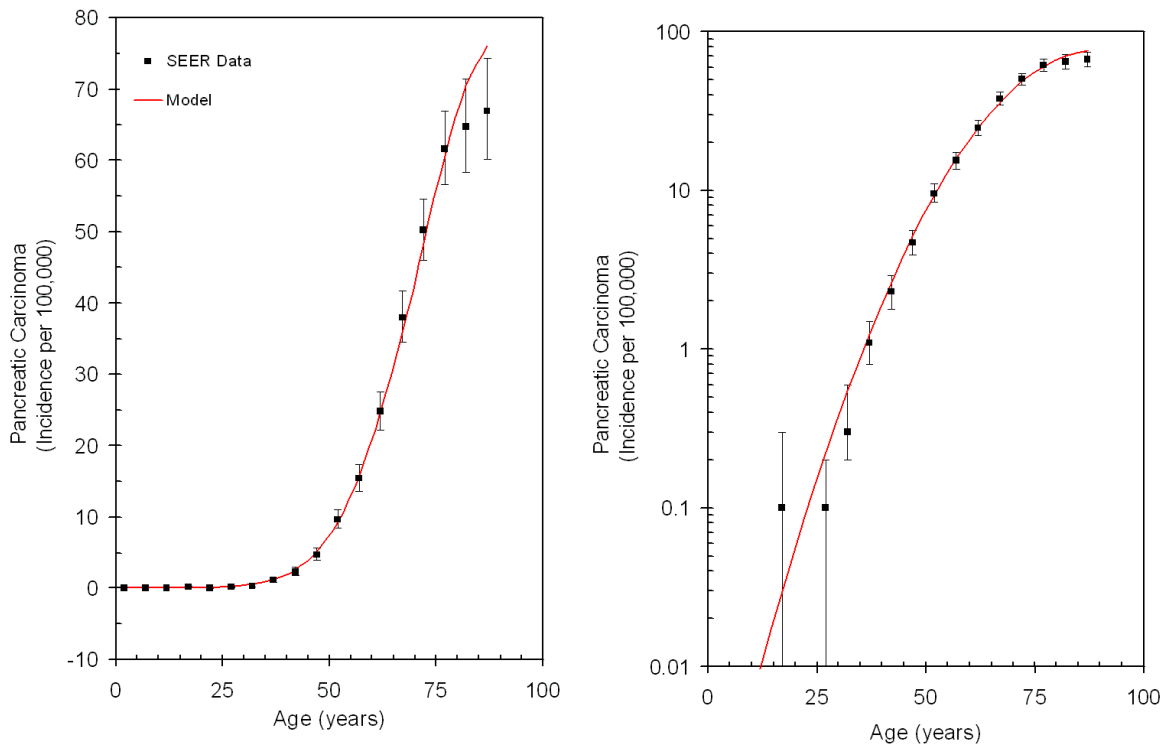


Figure 6: Supplemental Figure. The age-incidence of carcinoma of the pancreas. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.

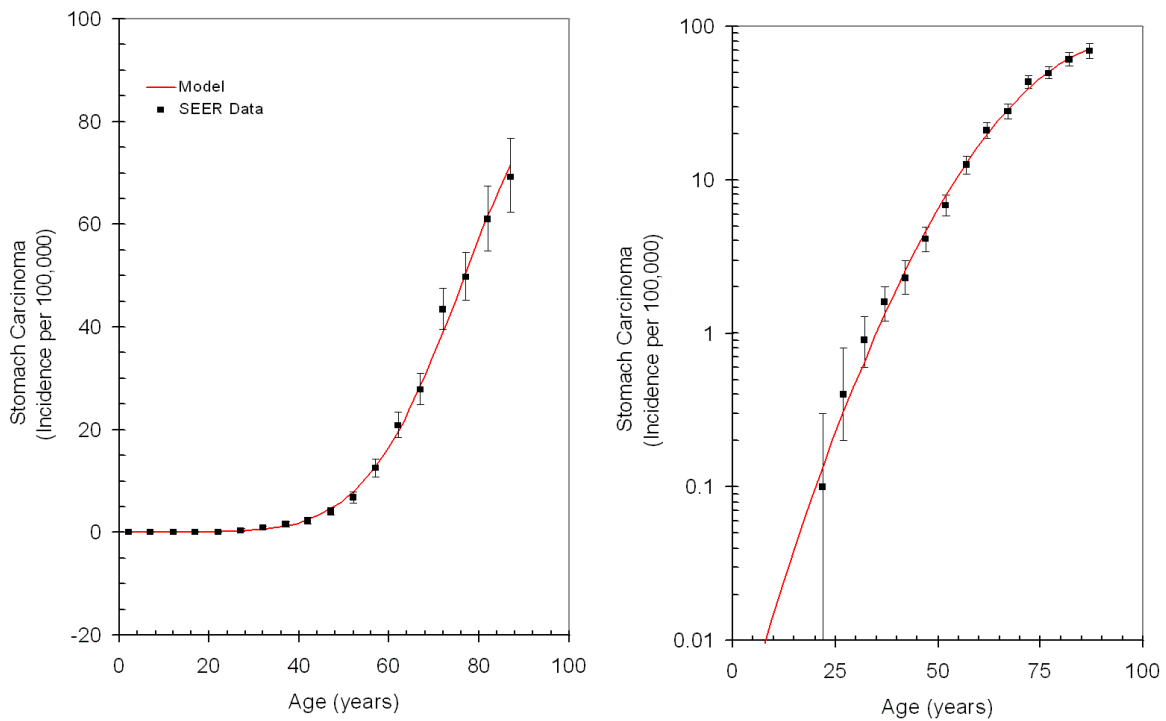


Figure 7: Supplemental Figure. The age-incidence of carcinoma of the stomach. As in Figure 2, the left and right panels show the same data. On the left the incidence is plotted on a linear scale, while on the right it is plotted on a logarithmic scale. In each case, the measured incidence is represented by a point and the 95% confidence intervals by error bars. The solid lines represents predicted incidence levels based upon the model.